DISTRIBUTION, PREVALENCE, AND GENETIC CHARACTERIZATION OF BAYLISASCARIS PROCYONIS IN SELECTED AREAS OF GEORGIA

Emily L. Blizzard‡, Cheryl D. Davis†, Scott Henke‡, David B. Long‡, Christopher A. Hall§, and Michael J. Yabsley∗

Warnell School of Forestry and Natural Resources, University of Georgia, Athens, Georgia 30602. e-mail: emily.blizzard@gmail.com; myabsley@uga.edu

ABSTRACT: Baylisascaris procyonis is an intestinal nematode of raccoons (Procyon lotor) that can cause fatal larval migrans in numerous species of birds and mammals, including humans. Although this parasite has historically been absent in the southeastern United States, it has been found in isolated regions in the Appalachian Mountains and was recently documented in Dekalb County, Georgia. The first objective of the current study was to investigate the distribution and prevalence of B. procyonis in selected populations of raccoons in Georgia. Intestinal tracts of 312 raccoons from 25 Georgia counties were examined for B. procyonis. The only county where B. procyonis was detected was Clarke County, where 12 of 116 (10.3%) raccoons were infected. In Clarke County, significantly more juveniles (P = 0.049) were infected compared with adults, and no differences in prevalence were noted by sex, season of capture, or land use (rural vs. urban); however, significantly (P = 0.0370) higher worm burdens were found in infected raccoons from urban/suburban locations compared with rural areas. In addition, Toxascaris leonina, a morphologically similar ascarid, was found in 3 raccoons from Clarke County (n = 2) and Morgan County (n = 1). A second objective was to determine if sequence polymorphisms were associated with B. procyonis from different geographic regions. Because sequences from a single worm from Japan had been entered into GenBank, we obtained nematodes from Kentucky and Texas for comparison with our samples from Georgia. Sequence analysis of the 18S and 5.8S rRNA genes and the internal transcribed spacer (ITS) -1 and ITS-2 regions confirmed Georgia sequences were B. procyonis. Although several polymorphic bases were observed within both ITS regions, none was associated with a particular geographic location. These data indicate that the distribution of B. procyonis within Georgia is increasing and only limited genetic variation is present in the rRNA and ITS gene regions among B. procyonis from the southern United States and introduced populations in Japan.

Increased anthropogenic changes to natural landscapes during recent decades has led to substantial increases in raccoon (Procyon lotor) population densities in some areas (Page et al., 2008). These heterogeneous landscapes, which are closely associated with humans, provide readily available food sources that raccoons commonly exploit (Lotze and Anderson, 1979; Riley et al., 1998; Gehrt, 2003). Raccoon densities in such urbanized areas can range from 37 to 333/km² compared to typical rural population densities of 2 to 20/km² (Lotze and Anderson, 1979; Riley et al., 1998; Gehrt, 2003; Prange et al., 2003). As anthropogenic change increases, more frequent interactions between animals and humans will occur. Combined with increasing raccoon populations, the risk for the transfer of zoonotic diseases could increase significantly. Increased density and interactions with other animals can result in increased transmission of numerous pathogens, including B. procyonis (Wright and Gomper, 2005; Page et al., 2009). Additionally, the illegal transport of raccoons can result in the introduction of novel diseases into new geographic areas (Nettles et al., 1979).

Although large numbers of B. procyonis adults may be present in the small intestine of raccoons, little to no disease has been reported (Carlson and Nielsen, 1984). In addition, domestic dogs can serve as definitive hosts (Bowman et al., 2005). When paratenic hosts, including humans, ingest mature eggs, migrating larvae can cause severe visceral, ocular, and neural larval migrans. Over 90 species of birds and mammals are susceptible to infection, and such infection often results in high morbidity or mortality of certain species of rodents, lagomorphs, and birds (Kazacos, 2001). In raccoons, the highest prevalences of B. procyonis are found in the northeastern, midwestern, and mid-Atlantic states, and along coastal areas of Texas, California, Washington, and Oregon (Kazacos, 2001). In the Southeast, this parasite has historically been restricted to the Appalachian regions of West Virginia and Virginia and isolated regions of Kentucky and Tennessee and a single infected raccoon from a northeast Georgia mountainous county during 1976 (Harkema and Miller, 1962; Jacobson et al., 1976; Bafundo et al., 1980; Schaffer et al., 1981; Jones and McGinnes, 1983; Smith et al., 1985; Cole and Shoop, 1987; Kazacos, 2001; Owen et al., 2004; Souza et al., 2009). In 2002, B. procyonis was detected in 22% (11/50) of free-ranging raccoons from Dekalb County, Georgia (Eberhard et al., 2003). Concurrent with that finding, a wildlife rehabilitator in Clarke County, Georgia reported finding B. procyonis in a single raccoon, although the history of this animal is unknown, e.g., county of origin (Eberhard et al., 2003). Because of the zoonotic and wildlife health implications of this parasite, we initiated this study to determine the prevalence and distribution of B. procyonis in selected counties of Georgia. In addition, we analyzed samples of B. procyonis from Georgia, Texas, and Kentucky to determine if genetic polymorphisms were present in the 18S and 5.8S rRNA genes and internal transcribed spacer (ITS) -1 and ITS-2 regions.

MATERIALS AND METHODS

Sample collection

From February 2005 to September 2009, 228 raccoons were collected from 4 northeastern Georgia counties (Clarke, Morgan, Oglethorpe, and Oconee) and 4 southern or coastal counties (Baker, Camden, Chatham, and Long) (Table I). All animals were either captured in box traps (Tomahawk Live Trap Co., Tomahawk, Wisconsin) baited with sardines or canned cat food, or were fortuitous road-kill collections.

All capture and handling procedures were approved by the UGA Institutional Animal Care and Use Committee. Captured raccoons were anesthetized with an intramuscular injection of ketamine hydrochloride...
RESULTS

Survey

Baylisascaris procyonis was detected in 12 of 312 (3.8%) raccoons in Georgia; all of the positives were from Clarke County (n = 116, 10.3%) (Fig. 1, Table I). Identification of these adult and immature worms was confirmed based on morphologic characteristics, or sequence analysis, or both. Among the infected raccoons, a range of 1–22 worms (average intensity 6.9 ± 8.2) was detected. Significantly more juvenile raccoons (P = 0.049) and raccoons from nonrural locations (P = 0.0370) were infected (Table II) compared with adults and raccoons from rural locations. No difference in prevalence was noted between sexes (P = 0.7601) or between seasons (all P values > 0.1). No differences in nematode intensity were observed between age, sex, or season classes; however, significantly (F = 5.78, P = 0.0370) higher worm burdens were found in infected raccoons from urban/suburban locations in Clarke County (Table II). Two raccoons from Clarke County and 1 from Morgan County were infected with another ascarid, Toxascaris leonina.

Genetic characterization

Sequences of the 18S rRNA (1,951 base pairs [bp]) and 5.8S rRNA (157 bp) genes from Georgia samples were identical to B. procyonis (GenBank U94368 and AJ001501, respectively). Only limited nucleotide polymorphisms were noted in the ITS-1 and ITS-2 regions (Tables III, IV). ITS-1 sequences from Georgia, Kentucky, and Texas were 99.3–98.8%, 99.3%, and 99.3–99.8%, respectively, identical to a B. procyonis sample from Japan (AB053230) (Table III). More sequence conservation was noted for the ITS-2 region with 10 samples from Georgia, Kentucky, and Texas being identical to a sample from Japan (AB051231) (Table IV). Single nucleotide polymorphisms (99.8% identity) were noted in 4 individual worms from Georgia (Table IV).

DISCUSSION

In Georgia, B. procyonis was first reported in a single raccoon (of 110, 0.9%) from a northeastern county in 1976 (Kazacos, 2001). Since that initial finding, additional surveys throughout Georgia failed to detect additional positives until 2002 when infected raccoons were detected in suburban Atlanta (DeKalb County) and a single raccoon with an unknown history admitted to a rehabilitation center in Clarke County (Jordan and Hayes, 1959; Harkema and Miller, 1962; Schaffer et al., 1981; Price and Haram, 1983; Eberhard et al., 2003). In the current study, we detected B. procyonis–infected raccoons only in Clarke County; however, low numbers of raccoons were surveyed in several of the tested counties. Currently, it is not known if the presence of B. procyonis in the Piedmont region of Georgia (DeKalb and Clarke Counties) is due to natural spread from endemic areas, e.g., from southern Tennessee (Souza et al., 2009), or possibly from translocation of infected raccoons from endemic areas. Historically, B. procyonis—infected raccoons in the southeastern United States were restricted to mountainous regions of West Virginia and Virginia and isolated regions of Kentucky and Tennessee (Harkema and Miller, 1962; Jacobson et al., 1976; Bafunzo et al., 1980; Schaffer et al., 1981; Jones and McGinnes, 1983; Smith et al., 1985; Cole and Shoop, 1987; Owen et al., 2004; Souza et al.,...
2009). A recent survey of raccoons from northwestern South Carolina failed to detect *B. procyonis* (Yabsley and Noblett, 1999), so additional surveys in northern Georgia are needed to establish if *B. procyonis* is present in mountainous regions of Georgia. In addition, a recent finding of *B. procyonis* in northwestern Florida raises concerns that this parasite might be present in southern Georgia (Blizzard, 2010).

Similar to numerous previous studies, we found that the prevalence of *B. procyonis* in raccoons from Clarke County was highest in juveniles (Snyder and Fitzgerald, 1985; Ermer and Fodge, 1986; Robel et al., 1989; Evans, 2001; Kazacos, 2001; Yeitz et al., 2009). Although prevalence rates are expected to be highest in juveniles because juveniles are more susceptible to infection by ingestion of eggs compared with adults (Kazacos, 2001), some studies in coastal Texas (Kerr et al., 1997), eastern Tennessee (Souza et al., 2009), and in British Columbia, Canada (Ching et al., 2000) failed to detect differences in prevalence between juveniles and adults. We did not detect a difference in prevalence between sexes similar to several other studies (Ermer and Fodge, 1986; Ching et al., 2000; Souza et al., 2009; Yeitz et al., 2009), although some studies have found higher prevalences in males (Snyder and Fitzgerald, 1985; Cole and Shoop, 1987; Snyder and Fitzgerald, 1987; Kidder et al., 1989; Robel et al., 1989; Evans, 2001; Wirsing et al., 2007). In the northeastern and midwestern United States, the prevalence of *B. procyonis* peaks in late summer and autumn and decreases during the winter (Smith et al., 1985; Kidder et al., 1989; Evans, 2001; Kazacos, 2001; Evans, 2002; Page et al. 2005); however, our results were similar

![Figure 1](image)

**TABLE I.** Prevalence of *Baylisascaris procyonis* in raccoons captured from 1997 to 2009 from 25 counties in Georgia.

<table>
<thead>
<tr>
<th>County(s)</th>
<th>Year(s)</th>
<th>n</th>
<th>No. positive (%)</th>
<th>Average intensity ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baker/Thomas</td>
<td>2003–2008</td>
<td>35</td>
<td>0</td>
<td>na*</td>
<td>na</td>
</tr>
<tr>
<td>Barrow/Ocone/Jackson</td>
<td>1997–2009</td>
<td>11</td>
<td>0</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>Chatham/Long</td>
<td>2006–2008</td>
<td>57</td>
<td>0</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>Clarke</td>
<td>2007–2008</td>
<td>116</td>
<td>12 (10.3)</td>
<td>6.9 ± 8.2</td>
<td>1-22</td>
</tr>
<tr>
<td>Columbia/Lincoln</td>
<td>1999–2001</td>
<td>2</td>
<td>0</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>Coweta</td>
<td>2005–2006</td>
<td>7</td>
<td>0</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>Dade/Floyd</td>
<td>2001–2009</td>
<td>49</td>
<td>0</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>Forsyth/Hall/Lumpkin/White</td>
<td>2001–2005</td>
<td>6</td>
<td>0</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>Glynn/McIntosh/Camden</td>
<td>2003–2008</td>
<td>17</td>
<td>0</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>Greene/Morgan/Walton</td>
<td>1997–2008</td>
<td>6</td>
<td>0</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>Madison/Oglethorpe</td>
<td>1999–2008</td>
<td>6</td>
<td>0</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>312</td>
<td>12 (3.8)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* na, not applicable.
to other studies conducted in other southeastern states (Tennessee, Kentucky, and Texas) where no difference in prevalence was noted between seasons (Smith et al., 1985; Kerr et al., 1997).

Our marginally significant higher prevalence in juveniles and lack of seasonal differences could be due to the current low prevalence in Clarke County, low numbers of raccoons surveyed, or the presence of a large number of naïve adult raccoons that are susceptible to infection. Also, a lack of extreme seasonal temperature changes in the southeastern United States could increase the period of raccoon activity and exposure to B. procyonis, which may limit seasonal fluctuations in prevalence (Smith et al., 1985; Kidder, 1989; Kerr et al., 1997). It has also been suggested that prevalence decreases during the winter because of a reduction in food resources in northern temperate regions in which animals may lose up to 50% of their total body mass when they experience extreme weather conditions for extended periods of time, during which time self-cure may occur (Stuewer, 1943; Mech et al., 1968; Kidder et al., 1989; LoGiudice, 1995). Additionally, it is also plausible that raccoons throughout the southern United States may rely more heavily on berries and fruits and less on carnivorous diets than their northern counterparts, which has been suggested in a study in Alabama, where the diet of raccoons from April to November primarily consisted of fruits and plant material (~50 to 80%) and infrequently consisted of vertebrates (Johnson, 1970). Alternatively, increased heat could increase desiccation at latrine sites, which may decrease the risk of young raccoons acquiring infections at latrine sites. The thermal tolerance of B. procyonis eggs in water has been determined to be >47°C; however, the effects of heat, UV exposure, and desiccation have not been studied (Shafrir et al., 2007). Collectively, these factors, i.e., less restrictive resource limitations, less substantial seasonal weight fluctuations during winter months, seasonal temperature differences, and decreased propensity to ingest infected intermediate hosts, could decrease transmission of B. procyonis; however, additional work is needed to confirm this hypothesis (Goldman, 1950).

In contrast to Page et al. (2008), we observed a trend toward higher prevalence and intensity of infection in suburban/urban areas compared with raccoons from nonurban areas of Clarke County, Georgia. Page et al. (2008) attributed the lower prevalence and lower nematode intensity in urban raccoons to behavioral factors, including change in foraging habits and differences in home range, which lead to a decreased exposure to B. procyonis eggs. Urbanized raccoons are more likely to forage in refuse and other human-provided food sources that would decrease the chances of a raccoon ingesting B. procyonis-infected paratenic hosts (Page et al., 2005, 2008). Furthermore, urbanized raccoons have been shown to have smaller home ranges, which limit contact and foraging in potentially contaminated areas (Gehrt, 2003; Prange et al., 2004; Page et al., 2008). In our current study, we classified raccoons as primarily low- and high-intensity urban or nonrural, based on dwelling or commercial property criteria. However, a high percentage of our nonurban lands (forest, sparse, pasture/crops) was in close proximity to urban areas, which suggests that raccoons in Clarke County likely forage across a range of habitat types. Furthermore, areas classified as urban or nonurban in Clarke County, Georgia are more similar to each other compared with the urban and nonurban areas examined by Page et al. (2008). Similar behavior and movement studies have not been conducted on raccoons from Clarke County, Georgia. Interestingly, we found significantly higher nematode intensity in infected raccoons from suburban/
urban areas compared with rural areas; however, only a few raccoons from the urban areas were infected with the highest worm burdens (n = 21 and 22 worms each), which may have influenced this observation.

In addition to B. procyonis, we detected T. leonina, which is a common parasite of domestic canids and felids, and several wild carnivore species, including raccoons in Texas and Saskatchewan, Canada (Wissing et al., 2007; Kresta et al., 2009). Careful morphologic examination of adult male nematodes or eggs from mature females is needed to distinguish these species definitively. In the current study, we detected several singleton immature infections, which complicated identification because distinguishing characteristics (roughened perianal patch on male B. procyonis or small cervical alae on T. leonina) are difficult or impossible to discern on immature nematodes.

Molecular characterization of the ascarids in this study served 2 purposes. First, it allowed definitive diagnosis of the worms, especially those that were immature. Second, it provided information on the genetic variability among our selected gene targets and geographic regions. For this work, we selected the 18S or 5.8S rRNA genes and the ITS-1 or ITS-2 regions, which have been used in previous studies to distinguish among Baylisascaris spp. and related ascarids (Zhu et al., 1998, 2001). We confirmed that the sequences of any of these genes or regions can be used to distinguish immature nematodes of the 2 morphologically similar ascarids that we found in the current study. The sequences we obtained from the 18S and 5.8S rRNA genes from B. procyonis from Georgia, Kentucky, and Texas and a sample from GenBank from Japan were identical. Although limited genetic variability was noted in the ITS-1 and ITS-2 regions, these polymorphisms were not associated with the any of the 4 locations from which B. procyonis was obtained. It would be interesting to obtain sequences from additional samples or gene targets of other B. procyonis endemic sites, e.g., western, upper midwestern, or northeastern United States, to determine if additional variation is present in these regions where B. procyonis was historically common.

ACKNOWLEDGMENTS

This work was supported by a grant from the Southeast Center for Emerging Biologic Threats and the Centers for Disease Control and Prevention to MJY. The authors thank D. Kavanaugh and J. Smith (APHIS/USDA/WS), C. Groce (WKU), and B. Hansen, K. Pederson, B. Wilcox, B. Adler, J. Carroll, G. Doster, S. Ellis-Felege, J. Gonyon, N. Jenkins, J. Slusher, J. Parris, W. Kistler, and B. Shock (UGA) for field assistance and/or permission to collect raccoons. In addition, we thank the various diagnosticians at SCWDS who conducted necropsies on clinical cases. CDD also gratefully acknowledges administrative support from NIH Grant Number 2 P20 RR-16481 from the National Center for Research Resources. Additional support was obtained from sponsorship of SCWDS member states.

LITERATURE CITED


